

Lipoprotein and Oxygen Transport Alterations in Passive Smoking Preadolescent Children

The MCV Twin Study

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We investigated the cardiovascular effects of lifelong passive cigarette smoke exposure in preadolescent children and examined the following questions: 1) Is systemic oxygen transport altered? 2) Are coronary heart disease risk factors adversely affected? We recruited 216 families from the MCV Twin Study; 105 had at least one smoking parent. Serum thiocyanate and cotinine levels were used as measures of smoke exposure in the children and thiocyanate was proportional to the number of parental cigarettes smoked each day ($p=0.0001$). Paternal smoking had no effect on these measures. Whole blood 2,3-diphosphoglycerate was higher in smoke-exposed than unexposed children ($p<0.01$) and was related to the thiocyanate level ($p<0.02$). High density lipoprotein (HDL) cholesterol was lower in passive smoking children ($p<0.05$); the HDL₂ subfraction was reduced in passive smoking boys, while the HDL₂ subfraction was reduced in passive smoking girls. Significant adverse alterations in systemic oxygen transport and lipoprotein profiles are already present in preadolescent children exposed to long-term passive cigarette smoke, primarily from maternal smoke. Children with long-term exposure to passive smoke may be at elevated risk for the development of premature coronary heart disease. (*Circulation* 1990;81:586-592)

The adverse health effects of actively inhaled cigarette smoke include impaired pulmonary function, increased coronary and cerebrovascular disease, chronic pulmonary disease, and cancer.¹⁻³ Cigarette smoking is a powerful independent risk factor for myocardial infarction, sudden death, peripheral vascular disease, and stroke and is the most important of the modifiable risk factors for coronary heart disease.⁴ The greatest relative risk related to smoking occurs in younger age groups,⁵ and an unusually high proportion of individuals with premature coronary heart disease are smokers.⁶ Therefore, smoking is an important risk factor associated with premature coronary heart disease.

Infants and young children of smoking parents who are passively exposed to cigarette smoke are more at risk for lower respiratory tract infections and small airway disease than are children of nonsmoking parents.^{7,8} What is less clear is whether the cardiovascular and oxygen transport systems of the growing child are adversely affected by long-term exposure to passive inhalation of cigarette smoke. Atherosclerotic changes found in middle-aged men may begin in childhood where certain risk factors are thought to be related to the earliest stages of atherosclerotic disease.^{9,10} Therefore, we asked the following questions: 1) Is systemic oxygen transport altered in chronically exposed passive smoking children of active smoking parents? 2) If abnormalities exist, are they related to the amount of cigarette smoke exposure? 3) Does passive cigarette smoking in preadolescent children detrimentally alter their coronary heart disease risk factors? To answer these questions, we evaluated the systemic oxygen transport variables, coronary risk factors, and echocardiographic cardiovascular measurements of 216 pairs of preadolescent twins from smoking versus nonsmoking families.

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Methods

Population

As part of an ongoing genetic longitudinal study of developmental changes in cardiovascular risk factors during adolescence, we recruited families with twins from nearby school systems. Eleven-year-old twins were ascertained from more than 75 middle schools of central Virginia within a 150-mile radius with use of a computerized population-based registry. Information packets were mailed to the schools for distribution to parents of twins to maintain confidentiality from the investigators. The parents who replied by mail (50%) were invited to participate.

The families participated in a protocol that included the collection of data on family health histories, smoking history (historical data provided by parents), blood pressure, electrocardiographic measurements, echocardiographic measurements, and the collection of blood samples for biochemical assays. The number of cigarettes smoked each day by the parents was recorded. No attempt was made to prescreen enrollees for the presence or absence of cardiovascular risk factors. Informed written consent, which had been approved by the Committee on the Conduct of Human Research of the Virginia Commonwealth University, was obtained from each family before it entered the study.

Procedures

Anthropometrics and blood pressure. Height and weight of each subject in stocking feet were measured with a stadiometer and digital scale, respectively. Sexual maturation was self-assessed by asking each subject to select a drawing of the Tanner stage of pubic hair development that most closely corresponded to his or her own level of sexual development.¹¹ Two resting blood pressure measurements were obtained with the subject in a sitting position using a mercury sphygmomanometer and the appropriately sized compression cuff. The fourth Korotkoff phase was recorded as the diastolic blood pressure.

Echocardiography. Echocardiographic left ventricular wall thicknesses and chamber dimensions were measured according to standardized measurement criteria.¹² Echocardiograms were obtained with the subject in the recumbent position using an SKI ultrasonoscope 20'A with a 3.5 MHz probe and Honeywell 1856 strip-chart recorder. Echocardiograms were obtained and read in a blinded fashion; the individuals performing and reading the echocardiograms were not aware of the passive smoking status of the children. The echocardiographic tracings were placed over a bit pad and using a microcomputer, digitized echocardiographic dimensions, wall thicknesses, and heart rate were measured and stored on diskette. The measurements were not adjusted for heart rate. The data from the diskette were transferred to a computer where the echocardiographic-derived variables were calculated.¹³

Blood samples. A sample of whole blood was obtained, stored on ice, and processed within 1 hour for quantitative lipoprotein cholesterol measurements using the vertical spin ultracentrifugation technique.¹⁴ Quantitative lipoprotein cholesterol levels were obtained on all but five nonsmoking and three passive smoking twin pairs. Hematocrit was determined in duplicate by capillary tube centrifugation. Early in the study, we obtained the techniques to measure whole blood thiocyanate level ($n=108$ twin pairs) and red blood cell 2,3-diphosphoglycerate level (2,3-DPG) ($n=163$ twin pairs). Blood thiocyanate concentration was determined by a quantitative colorimetric method at 450 nm^{15,16} and red cell 2,3-DPG level was determined by the method of Fiske and SubbaRow.¹⁷ Serum cotinine concentration was quantitated by radioimmunoassay methods.^{18,19}

Data Analysis

Data are presented as mean \pm SD. Statistical differences between group means were assessed by two-sided t tests, taking into account whether group variances were equal. Because twins share genes and environments and represent nonindependent observations, data from only a single twin randomly ascertained from each family was used to determine group means for statistical testing. Nonparametric correlation coefficients using the Kendall Tau B statistic were used when it was apparent that a given variable was not normally distributed, such as cigarettes smoked each day, serum thiocyanate, and high density lipoprotein (HDL) cholesterol. Regression analysis was used to remove the effects of confounding variables.

Group means for passive smoking and nonsmoking subjects were adjusted to correct for differences in age, height, weight, and, when the groups included both males and females, sex. A multiple linear regression analysis was conducted in which the response variable was modelled as a linear function of the above covariates. Regression coefficients were obtained and the expected value of the response variable was calculated with the covariates fixed to their mean values. These adjustment computations were carried out using the LSMEANS option of the General Linear Models procedure of the SAS statistical package. The heritability of specific variables was estimated as two times the difference of the twin correlations in monozygotic and dizygotic pairs.²⁰ All results were considered statistically significant at $p < 0.05$.

Results

Smoking data were available on 216 families enrolled in the MCV Twin Study. One hundred eleven of these families had nonsmoking parents. Of these nonsmoking families, both parents were never smokers in 50, the father smoked in the past in 25, the mother smoked in the past in nine, and in 27 both parents smoked in the past. Of the mothers who smoked in the past, 21 smoked during the pregnancy of the twins. Fathers who smoked in the past stopped

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smoking 10.0 ± 6.7 years before evaluation, though five stopped smoking within 1 year of the study. Mothers who smoked in the past stopped smoking 8.6 ± 7.3 years before evaluation with seven stopping within 1 year of the study.

In 105 families, either or both parents were cigarette smokers at the time of evaluation, and maternal smoking during pregnancy occurred in 69 of these. In the 105 smoking families, the father was the only smoker in 44%, the mother in 32%, and both parents were smokers in 24%. The fathers began smoking at 18.2 ± 6.2 years of age and presently smoke 24.5 ± 12.6 cigarettes/day. The mothers began smoking at 18.4 ± 4.3 years of age and presently smoke 18.5 ± 9.7 cigarettes/day. The total daily number of cigarettes smoked by the parents ranged from 1 to 10 in 17%, 11 to 20 in 32%, and was greater than 20 in 51%.

Data were obtained and analyzed on 105 passive smoking twin pairs and 111 non-passive smoking twin pairs. Of the non-passive smoking twin pairs, 61 were monozygotic and 50 were dizygotic, while of the passive smoking twin pairs, 55 were monozygotic and 50 were dizygotic. None of the twins had ever smoked cigarettes.

Indexes of passive cigarette smoke exposure were obtained by measuring serum levels of cotinine and thiocyanate. The passive smoking twins ($n=35$) demonstrated higher levels of thiocyanate than the non-passive smoking twins ($n=89$) (7.1 ± 4.3 vs. 3.1 ± 3.0 mg/L, $p < 0.0001$). Passive smoking boys and girls had similar elevations of thiocyanate (7.0 ± 4.1 and 7.3 ± 4.5 mg/L, respectively). Cotinine was not detected in non-passive smoking twins but was present in passive smoking twins (1.5 ± 3.1 ng/ml), and serum thiocyanate level correlated with the cotinine level ($r=0.44$, $p < 0.005$). The level of thiocyanate in non-passive smoking twins is best explained by non-tobacco dietary sources of thiocyanate as we can exclude the possibility of significant smoke exposure outside their homes due to the absence of cotinine in their blood. The intrawin pair correlation for thiocyanate was high ($r=0.94$, $p < 0.0001$), demonstrating that twins within a smoking family generally have similar exposure to home environmental cigarette smoke.

Within all smoking families, thiocyanate level correlated with the total number of cigarettes smoked each day ($r=0.35$, $p < 0.0001$). In a subgroup of smoking families in which the mother but not the father smoked ($n=14$), there was good correlation between thiocyanate level in the twins and the number of cigarettes smoked each day by the mother ($r=0.57$, $p < 0.01$) (Figure 1), whereas in families in which the father was the only smoker ($n=38$), no correlation was found. This suggests that paternal cigarette smoking provides little or no contribution to the home passive smoking environment and that maternal cigarette smoking is the major source of child-home passive smoke exposure.

The unadjusted data on passive smoking and non-passive smoking groups as a whole and separated by

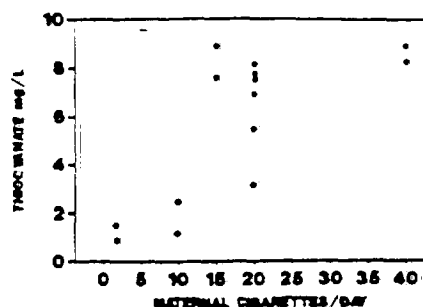


FIGURE 1. Plot of the relation of child serum thiocyanate level to number of cigarettes smoked each day by the mother. Plotted points represent data from 14 passive smoking children. Kendall Tau B correlation coefficient = 0.57, $p < 0.01$.

sex are presented in Table 1, while variables of interest after adjustment for age, height, weight, and sex are presented in Table 2. Passive smoking and nonsmoking groups were similar for age, Tanner stage, height, systolic blood pressure, and diastolic blood pressure. Girls were more advanced in sexual development by Tanner stage than boys in both non-passive smoking and passive smoking groups ($p < 0.01$). Passive smoking children weighed slightly more than non-passive smoking children.

The hematologic data on passive smoking and non-passive smoking groups are shown in Tables 1 and 2. The mean hematocrit value was similar for the two groups. Passive smoking children had higher whole blood levels of 2,3-DPG. While this difference was significant in the boys, a similar trend was present in the girls. In smoking families, the 2,3-DPG level correlated directly with the serum thiocyanate level and the total number of cigarettes smoked by the parents (both, $p < 0.05$). The relation between the 2,3-DPG level and the serum thiocyanate level in passive smoking children ($r=0.29$, $p < 0.02$) is shown in Figure 2.

Quantitative lipoprotein cholesterol levels are presented in Tables 1 and 2. The mean time elapsed from the last meal to the time of blood drawing was 6.3 hours and was similar for passive smoking and non-passive smoking groups. In our population, the duration of fasting did not contribute to the variance of either total cholesterol or lipoprotein levels.²¹ The passive smoking group had significantly lower total cholesterol than the non-passive smoking group. Passive smoking boys had slightly higher total cholesterol and low density lipoprotein (LDL) cholesterol levels than non-passive smoking boys, though these differences were not statistically significant. However, passive smoking girls had significantly lower levels of total cholesterol and LDL cholesterol when compared with non-passive smoking girls.

Significant intergroup differences were seen in the HDL cholesterol subfractions. Total HDL cholesterol was lower in the passive smoking group when compared with the non-passive smoking group, even after adjusting for age, weight, height, and sex. This

TABLE 1. Unadjusted Mean \pm SD for Passive Smoking and Nonsmoking Twin Groups

	All twins		Boys		Girls	
	Nonsmoking (n=111)	Passive smoking (n=105)	Nonsmoking (n=56)	Passive smoking (n=50)	Nonsmoking (n=55)	Passive smoking (n=55)
Age	11.8 \pm 1.2	11.9 \pm 1.2	12.0 \pm 1.4	11.8 \pm 1.1	11.6 \pm 1.0	11.9 \pm 1.3
Tanner	2.6 \pm 1.2	2.7 \pm 1.3	2.5 \pm 1.2	2.4 \pm 1.2	2.8 \pm 1.3	3.0 \pm 1.3
Height (cm)	149.1 \pm 9.9	150.5 \pm 9.1	150.0 \pm 11.4	149.8 \pm 9.1	148.2 \pm 8.2	151.1 \pm 9.1
Weight (kg)	39.8 \pm 8.9	43.1 \pm 11.3*	40.1 \pm 9.1	43.2 \pm 11.8	39.6 \pm 8.8	43.0 \pm 10.9
Heart rate (beats/min)	72.9 \pm 12.5	72.6 \pm 12.4	67.7 \pm 9.2	69.1 \pm 10.5	77.7 \pm 13.3	76.2 \pm 13.3
SBP (mm Hg)	106.9 \pm 9.9	109.1 \pm 9.6	106.3 \pm 10.5	110.1 \pm 10.6	107.5 \pm 9.2	108.2 \pm 8.6
DBP (mm Hg)	59.5 \pm 11.5	61.5 \pm 10.8	57.8 \pm 11.4	61.0 \pm 13.0	61.2 \pm 11.4	62.0 \pm 8.6
Hematocrit (%)	39.8 \pm 1.9	39.5 \pm 2.2	40.0 \pm 1.9	40.0 \pm 2.5	39.6 \pm 1.8	39.1 \pm 1.8
DPG (μ m/ml)	1.98 \pm 0.28	2.08 \pm 0.23*	1.89 \pm 0.26	2.08 \pm 0.23*	2.05 \pm 0.27	2.08 \pm 0.24
Cholesterol (mg/dl)	172.8 \pm 24.8	164.3 \pm 29.5*	168.2 \pm 22.0	170.1 \pm 31.2	177.4 \pm 26.8	158.6 \pm 26.8
LDL (mg/dl)	86.5 \pm 19.5	81.7 \pm 21.8	81.6 \pm 17.8	85.0 \pm 23.2	91.4 \pm 20.1	78.5 \pm 20.0
HDL (mg/dl)	49.5 \pm 9.3	45.7 \pm 10.4†	49.3 \pm 8.8	45.2 \pm 9.6*	49.7 \pm 9.7	46.1 \pm 11.2
HDL ₂ (mg/dl)	13.9 \pm 7.4	12.1 \pm 7.1	13.6 \pm 7.2	10.8 \pm 6.3*	14.2 \pm 7.6	13.2 \pm 7.7
HDL ₃ (mg/dl)	35.7 \pm 6.5	33.6 \pm 5.8*	35.8 \pm 5.4	34.4 \pm 5.8	35.5 \pm 7.6	32.8 \pm 5.8*
L/H	1.80 \pm 0.52	1.88 \pm 0.67	1.70 \pm 0.47	1.95 \pm 0.64*	1.90 \pm 0.55	1.81 \pm 0.69
LVM (g)	90.8 \pm 18.5	99.1 \pm 21.5*	96.8 \pm 19.1	104.7 \pm 20.7	85.4 \pm 16.4	93.9 \pm 21.4

DBP, diastolic blood pressure; DPG, 2,3-diphosphoglycerate; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; L/H, LDL/HDL ratio; LVM, left ventricular mass; SBP, systolic blood pressure.

* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$.

comparison is shown in Figure 3. The LDL/HDL ratio was significantly elevated in the passive smoking boys, though the difference lost significance ($p = 0.06$) after the data were adjusted. The HDL₂ cholesterol subfraction level was consistently lower in all the passive smoking groups but this difference reached significance only for the unadjusted levels in the boys. An inverse trend was found between the total number of cigarettes smoked daily by the mothers and the serum HDL cholesterol level in the children. The lowest HDL₂ cholesterol levels were found in boys exposed to the highest number of cigarettes smoked daily by their mothers. These differences however did not meet statistical significance. The HDL₃ subfraction was significantly lower in the passive smoking group than the non-passive smoking group, with greater differences seen in the girls.

Because of the observed influence of maternal but not paternal cigarette smoking on oxygen transport and lipoprotein profiles, we investigated the possibility that maternal smoking may have affected children during gestation. We therefore compared the data adjusted for age, height, weight, and sex obtained on one twin per family who never had exposure to cigarette smoke ($n = 33$) to that of twins exposed only during gestation by maternal smoking ($n = 8$). An effect of fetal exposure on the HDL cholesterol level was found with lower HDL cholesterol levels in those children exposed in utero (44.6 ± 2.2 vs. 50.2 ± 1.1 mg/dl, $p < 0.05$), though the sample size was quite small. No other significant differences were found between these groups.

Echocardiograms suitable for measurement were obtained on 74 non-passive smoking and 66 passive smoking twin pairs. Left ventricular internal dimen-

TABLE 2. Mean \pm SD in Passive Smoking and Nonsmoking Twin Groups After Adjustment for Age, Weight, Height, and Sex

	All twins		Boys		Girls	
	Nonsmoking (n=111)	Passive smoking (n=105)	Nonsmoking (n=56)	Passive smoking (n=50)	Nonsmoking (n=55)	Passive smoking (n=55)
DPG (μ m/ml)	1.97 \pm 0.03	2.09 \pm 0.03‡	1.90 \pm 0.04	2.08 \pm 0.04‡	2.03 \pm 0.04	2.10 \pm 0.04
Cholesterol (mg/dl)	172.2 \pm 2.7	164.1 \pm 2.7*	168.9 \pm 3.7	169.8 \pm 3.7	176.6 \pm 3.7	157.6 \pm 3.7‡
LDL (mg/dl)	86.1 \pm 2.0	81.3 \pm 2.0	81.8 \pm 2.8	84.7 \pm 2.9	91.0 \pm 2.7	77.5 \pm 2.7‡
HDL (mg/dl)	49.1 \pm 0.9	46.0 \pm 0.9*	49.1 \pm 1.3	45.5 \pm 1.3	49.2 \pm 1.4	46.4 \pm 1.4
HDL ₂ (mg/dl)	13.5 \pm 0.7	12.5 \pm 0.7	13.2 \pm 0.9	11.3 \pm 0.9	13.9 \pm 1.0	13.5 \pm 1.0
HDL ₃ (mg/dl)	35.6 \pm 0.6	33.5 \pm 0.6*	35.9 \pm 0.8	34.1 \pm 0.8	35.3 \pm 0.9	32.8 \pm 0.9
L/H	1.81 \pm 0.05	1.86 \pm 0.06	1.72 \pm 0.08	1.94 \pm 0.08	1.90 \pm 0.55	1.81 \pm 0.69
LVM (g)	93.6 \pm 1.7	95.9 \pm 1.8	100.9 \pm 2.6	100.2 \pm 2.8	87.3 \pm 2.4	91.4 \pm 2.5

DPG, 2,3-diphosphoglycerate; LDL, low density lipoprotein; HDL, high density lipoprotein; L/H, LDL/HDL ratio; LVM, left ventricular mass. * $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$.

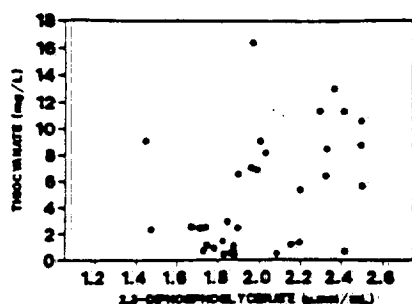


FIGURE 2. Plot of the relation between whole blood 2,3-diphosphoglycerate level and the serum thiocyanate level in passive smoking children ($n=35$): Kendall Tau B correlation coefficient = 0.29, $p < 0.02$.

sions in systole and diastole were the same for the two groups. The passive smoking group was found to have a higher left ventricular mass than the nonsmoking group, though the difference was lost after the data were adjusted for body size (Tables 1 and 2).

Covariates of smoking behavior and other confounding variables were considered, which could have affected the results. When parental income, education level, years of education, and beer and liquor consumption were compared between parents in smoking and nonsmoking families, no differences were found.

When we compared the exercise level (the number of times each week vigorous exercise was performed) in the twins themselves, the number of exercise episodes each week were similar for passive smoking and non-passive smoking boys (4.7 ± 2.0 vs. 4.2 ± 2.3 times/wk) and for passive smoking and non-passive smoking girls (4.3 ± 2.3 vs. 4.7 ± 2.1 times/wk). χ^2 tests showed no association between smoking status and exercise in either the boys ($\chi^2 = 1.7$, $p < 0.2$) or the girls ($\chi^2 = 0.5$, $p < 0.5$).

A preliminary estimate of the heritability of specific variables was obtained using the study's twin

TABLE 3. Intrapair Twin Correlations and Heritability

	Monozygotic ($n=116$)	Dizygotic ($n=100$)	Heritability
WT	0.91	0.57	68%
SBP	0.66	0.33	66%
SCN	0.94	0.91	6%
DPG	0.65	0.41	48%
LDL	0.81	0.35	92%
HDL	0.81	0.42	78%
HDL ₂	0.81	0.36	88%
HDL ₃	0.53	0.50	6%

All monozygotic twin correlations are significant at $p < 0.0001$. All dizygotic twin correlations are significant at $p < 0.005$.

WT, weight; SBP, systolic blood pressure; SCN, thiocyanate; DPG, 2,3-diphosphoglycerate; LDL, low density lipoprotein; HDL, high density lipoprotein.

design. Intrapair twin correlations for identical and nonidentical twins are shown in Table 3. The heritability is indicative of the variation attributable to genetic effects. The correlation for identical twins is significantly higher than for nonidentical twins for all variables except serum thiocyanate and HDL₃ cholesterol levels.

Within sampling error, the monozygotic correlation is twice the dizygotic correlation for systolic blood pressure, HDL cholesterol, HDL₂ cholesterol, LDL cholesterol, weight, and left ventricular mass. These values are expected if mating is random with respect to the causes of juvenile measures, gene action is additive, and family environment does not cause twin resemblance. These data also indicate that a high proportion of the variation in thiocyanate and HDL₃ cholesterol levels is attributable not to genetic effects but to environmental effects, such as passive smoking. The variation in 2,3-DPG levels appears balanced between genetic and environmental effects.

Discussion

We found alterations in systemic oxygen transport and lipoprotein composition in preadolescent children that were related to cigarette smoke exposure. Paternal smoking did not influence measures of passive smoke exposure, while maternal smoking affected children by providing passive smoke exposure in the home and possibly during gestation.

Our results indicate that, as in other tissue hypoxia states (anemias, chronic pulmonary disease, cyanotic heart disease, and high altitude), the body attempts to compensate for hypoxia by increasing the 2,3-DPG level in the blood to meet tissue oxygen requirements. A hypoxia-driven mechanism to trigger 2,3-DPG synthesis may be responsible for the increase in 2,3-DPG level in active smokers.^{22,23}

Erythrocytosis occurs frequently in adult active smokers. Hematocrit elevation in active smokers has been ascribed to long-term exposure of even low levels of carbon monoxide, which results in tissue hypoxia and leads to increased red cell mass.²⁴ Hematocrit values for the passive smoking and non-

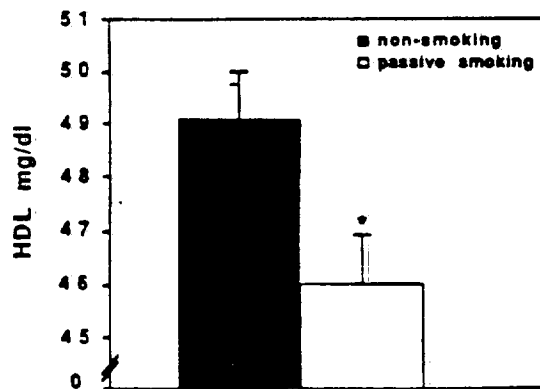


FIGURE 3. Bar graph of the comparison of total serum HDL cholesterol level in non-passive smoking ($n=106$) and passive smoking ($n=102$) children after adjusting for age, sex, height, and weight. Data represent group mean \pm SD. * $p < 0.05$. HDL, high density lipoprotein.

passive smoking children in the present study were identical, though both groups were in the early stages of pubertal development. Steroid and androgenic hormones, which positively influence erythropoiesis,²⁵ are low in preadolescent children and progressively increase during puberty. Longitudinal evaluation of passive smoking and non-passive smoking twins as they progress through puberty may detect differences in hematocrit and other oxygen transport variables, which may be related to the degree of passive cigarette smoke exposure.

The incidence of atherosclerotic coronary artery disease is strongly associated with increased levels of LDL cholesterol and decreased levels of HDL cholesterol, especially the HDL₂ cholesterol subfraction.²⁶⁻²⁷ Active cigarette smoking alters the total serum cholesterol concentration and lipoprotein composition, which directly increase the risk of coronary heart disease. In our population, children with a family history of premature cardiovascular death had lower levels of HDL₂ cholesterol than those without such a history.²¹

During puberty and early adolescence, levels of HDL and LDL cholesterol decrease in all children, but the decrease in HDL cholesterol is more pronounced in boys than in girls.²⁸ Since the girls in our study population were more sexually developed than the boys, we cannot exclude the possibility that the passive smoking girls were more advanced in pubertal development than their non-passive smoking counterparts, which could explain the observed differences in LDL cholesterol. HDL cholesterol levels fall during puberty in boys in association with increases in testosterone levels.²⁹ Passive cigarette smoking, by further diminishing the level of HDL₂ cholesterol in pubertal males, may be associated with accelerated atherosclerotic changes and an increased risk of coronary heart disease.

The passive smoking preadolescent boys demonstrated a tendency toward lower levels of the HDL₂ cholesterol subfraction, which was related to the number of cigarettes smoked daily by the parents of the boys. Because Bodurtha et al.²¹ showed that coronary heart disease deaths occur more frequently in families with low levels of HDL₂ cholesterol, a lower HDL₂ cholesterol level in passive smoking boys likely represents an enhanced atherogenic risk factor for the subsequent development of atherosclerotic coronary heart disease.

Haffner et al.³⁰ found a reduction in HDL₂ cholesterol subfraction levels with active cigarette smoking. These authors also found that alcohol consumption raised HDL₂ cholesterol levels. It appears therefore that HDL₂ cholesterol levels represent a reactive lipoprotein species that responds to specific environmental influences. Our data support this hypothesis by not only demonstrating lower HDL₂ cholesterol levels in passive smoking children but also the low heritability of HDL₂, implying high environmental variance.

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